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Two weeks later a second series of inoculations was performed, using three rabbits of the first series, three normal controls, and two other previously vaccinated rabbits. The material was the same as before except that suspensions of crusts from lesions produced on a monkey by Haitian and Jamaican G alastrim were substituted for the original (human) Haitian and Jamaican H materials. Fresh vaccine virus was also used. Possibly on account of the age of the material, negative or doubtful reactions were observed, except with the fresh vaccine virus. The chicken pox was, however, consistently negative.

Results: Smallpox crusts, vaccine virus, and alastrim material produced positive intracutaneous reactions in animals previously immunized to vaccine virus. Chicken pox did not produce such reactions.

Summary of Results.

A vesico-papular eruption was produced in monkeys by inoculation both with crusts and with vesicle contents from alastrim patients. The animals were protected against reinoculation with alastrim and vaccine virus. Rabbits inoculated with alastrim showed no eruption, but were almost completely immune to vaccine virus. Rabbits previously inoculated with vaccine virus gave positive intracutaneous reactions to smallpox crusts, alastrim material, and vaccine virus, but remained negative to chicken pox crusts.

The fact that definite immunity to vaccinia is produced by previous inoculation with alastrim is additional evidence of the essential identity of alastrim with smallpox.

INHALATION EXPERIMENTS ON INFLUENZA AND PNEUMONIA, AND ON THE IMPORTANCE OF SPRAY-BORNE BACTERIA IN RESPIRATORY INFECTIONS.[1]

By WILLIAM B. WHERRY, Surgeon (Reserve), and C. T. BUTTERFIELD,[2] Assistant Bacteriologist, United States Public Health Service.

During the epidemic of influenza occurring in the winter season of 1919–20, the junior author was assigned by the Public Health Service to cooperate with the senior author in a study of this disease. The work was done in the Pathologic Institute of the University of Cincinnati. The institute is in direct connection with the Cincinnati General Hospital. This close association of the institute with the hospital presented a favorable opportunity for securing fresh

[1] From the Laboratory of Bacteriology and Hygiene, University of Cincinnati, and the United States Public Health Service.

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[2] Detailed to the work by the United States Public Health Service.

specimens early in the course of the disease, for studying the individual cases, and for securing post-mortem material when such material was available.

At the beginning, three lines of study were adopted: (1) A routine bacteriological examination of throat swabs, sputum, and blood from typical influenza and influenza-pneumonia patients; (2) attempts to transmit influenza to animals by spray-borne material and by other methods of exposure; and (3) a study on the importance of spray-borne bacteria in relation to pneumonic infections in general. As the results obtained suggested, from time to time, under divisions (2) and (3), various factors which might prevent infection in the animal or which might predispose the animal for infection were tried out.

ROUTINE BACTERIOLOGICAL EXAMINATIONS.

In the routine bacteriological examination of throat swabs, sputum, and blood specimens attention was given only to cases which appeared typical. Thirty-eight such cases were examined. The medium used for isolation was agar, reaction +0.5 to phenolphthalein and containing 5 per cent of fresh defibrinated rabbit's blood. All cultures were made in triplicate and were incubated at 37° C., under aerobic, partial tension oxygen, and anaerobic conditions. *B. influenzæ*-like organisms were isolated from 12 of the 38 cases. Six of the 12 (Table I, strains 1 to 6) were from one family, S., all of whom became ill at about the same time. From the father of this family we failed to isolate *B. influenzæ*, and he was ill for months with streptococcus empyema. In 4 of the cases from this family, *B. influenzæ* was present in the sputum in pure culture. Most of the other organisms isolated belonged to the pneumococcus, hemolytic streptococcus, and staphylococcus groups. Of the 38 cases, 31 harbored hemolytic streptococci, and in 17, the pneumococcus was the predominant species present. No colonies were encountered which showed any resemblance to those of the colon-typhoid-enteritidis groups.

No trouble was experienced in isolating *B. influenzæ* on our medium. When grown under partial tension oxygen conditions, it retained its minute bipolar form and showed less tendency to produce involution forms than when grown under aerobic conditions. None of our strains of *B. influenzæ* would grow unless hemoglobin in some form was present in the medium. The *B. influenzæ* colonies were small in size and dewdrop-like in appearance, unless they were growing in close proximity to colonies of staphylococci, when they grew much larger and tended to coalesce. The Koch-Weeks strains isolated by us have grown out on plain ascites agar containing no hemoglobin.

PREPARATION OF ANTIGENS AND SERA FOR AGGLUTINATION WORK.

Antigens were prepared for the agglutination work as follows: The medium used was meat infusion 2 per cent agar, adjusted to a reaction +0.5 to phenolphthalein. (For the influenza strains, 5 per cent of defibrinated rabbit's blood was added while the medium was at a temperature of about 90° C.) The medium was used as slants in half-pint Blake bottles. Twenty-four-hour slants of the organism from which antigen was to be made were washed off in 5 c. c. of broth. This amount of emulsion was used to seed the surfaces of two Blake bottle slants. The bottles were then placed in the incubator, and after allowing the broth emulsion to stand on the medium for about 2 hours, the bottles were inverted and incubated for 48 hours. They were then removed, and the remaining broth, together with any condensation water, was pipetted off and thrown away. Five c. c. of saline were then added to each bottle, and the growth was washed off. This emulsion was placed in a sterile test tube and killed immediately by heating at 65° C. for 30 minutes. During this time, smears of the emulsion were made and examined for purity. If not pure, they were discarded. The tubes were then centrifuged at high speed, the supernatant fluid was poured off, and fresh saline which contained 0.5 per cent of phenol was added. The emulsion was then diluted until it had a turbidity of 1,000 p. p. m. by the Fuller method of measuring turbidity. This procedure gave uniformly a good antigen.

Antisera for the agglutination work were prepared for the influenza and Koch-Weeks bacillus by inoculating rabbits with live cultures. In the case of the enteritidis-like organism, designated M–5, described later, killed cultures had to be used because of the high virulence of that organism for laboratory animals. Fairly low-titer sera, 1/800, were used in these tests.

Table I contains the serological reactions of the influenza strains with the "Mother" and Koch-Weeks antisera. The first 6 strains were isolated from the different members of a family (S.) of 10, all of whom had influenza and had become ill at approximately the same time. The seventh culture, listed C, was isolated from the sputum of a normal individual who did not have and had not had influenza in any form. The eighth strain, A, was isolated from a post-mortem lung, and was the only organism that we were able to cultivate from the specimen. The last culture, listed "Koch-Weeks," was isolated from a typical case of "pink-eye" and was added to the stock to obtain the serological comparison. The remaining strains listed were obtained from the sputa of isolated cases.

One observes from Table I that 4 of the strains from the family S. group, "Mother," B, S, and M, and the normal strain, "C," are

probably identical with each other. One other of the family strains, H, is somewhat related to this group, but is about as closely related to the Koch-Weeks strain. The other strains tested are serologically (agglutinin and agglutinin absorption test) entirely different from the "Mother" and the Koch-Weeks strains. This agrees with the work of others, Krumwiede et al., 1919 (Jour. Med. Res. 39, p. 449), who have established the facts that there is a relationship between the influenza strains and the Koch-Weeks bacilli, and that there are distinct subdivisions among the so-called *B. influenzæ* strains.

Where possible, specimens of blood for serological use were obtained from patients from whom cultures of *B. influenzæ* had been isolated. In no instance were we able to demonstrate agglutinins for the bacteria isolated from these cases.

TABLE I.—*B. influenzæ—Agglutination and absorption of agglutinin reactions.*

Strain.	Mother antiserum.		Koch-Weeks antiserum.	
	Simple agglutination.	Homologous agglutinins absorbed.	Simple agglutination.	Homologous agglutinins absorbed.
(1) Mother	+	+	−	
(2) B	+	+	−	
(3) H	+	P	+	P
(4) St	+	+	−	
(5) M	+	+	−	
(6) S	−		−	
(7) C	+	P	−	
(8) A	−		−	
(9) Sum	−		−	
(10) K	−		−	
(11) Leo	−		−	
(12) Koch-Weeks	−		+	+

NOTE.— +means positive agglutination; −means no agglutination; P means homologous agglutinins (those for antigen with which antiserum was made) are only partially absorbed. When simple agglutination tests were negative, absorption tests were not done.

ATTEMPTS TO TRANSMIT INFLUENZA TO WHITE MICE, RATS, GUINEA PIGS, AND RABBITS, BY MEANS OF SPRAYED SPUTUM.

While it is realized that the influenza virus appeared to be of reduced virulence during the outbreak of the spring of 1920, the occasional occurrence of a family outbreak, or of rapid death due to pulmonary edema, seemed to indicate that it was identical with the virus present in the outbreak of 1919. We feel particularly sure that we were dealing with true cases of influenza in the family S., where the mother, father, and 8 children all came into the hospital at once. Most of these 10 cases had broncho-pneumonia and one had purulent pleuritis. Cases R and S, B and B, and T were of a milder type, although B and T had secondary broncho-pneumonia. These cases had been ill for only a day or two at the time the experimental material was collected.

While the examinations into the bacteriology of the cases were being carried out, attempts were made to transfer the infection directly to animals. It was thought that greater success might be achieved in reproducing the disease in animals if they were exposed to the infectious material in the form of a fine spray in the air which they were breathing. By this method the virus would come in contact not only with the nose, eyes, mouth cavity, and trachea of the animal, but it would also be carried down into the finest air passages of the lungs, as is shown in later experiments.

To do this, a "spray chamber" was devised, with a door which could be sealed after the animals had been placed inside. In addition, the box was arranged with two openings, one through which the spray was introduced by forcing it in with an atomizer, and the other an air vent (fitted with a cotton filter) to allow the escape of the excess air pressure created by the introduction of the spray. The animals exposed were placed in this chamber, and the contained air was kept saturated with the spray for from 30 minutes to an hour. A glass window was provided for the cage so that one might observe the saturation of the atmosphere and the condition of the animals. Each series of animals was kept in a separate cage. The cages were scalded with hot water before they were used for any given series. The sawdust bedding was not sterilized. Precautions were taken to sterilize the drinking pans in the case of all mouse experiments. The animals were fed on cracked maize and vegetable waste from the hospital kitchen.[3]

In doing the following experiments, sputum, or, when this could not be obtained, material swabbed from the tonsillar area, but generally both, were thoroughly shaken with 0.9 per cent salt solution and sprayed into the "spray chamber" by means of a De Vilbiss atomizer, within half an hour after the collection of the specimen.

The data of the sputum-spray experiments and experiments resulting from the death of sputum-spray animals, is compiled chronologically in Table II.

[3] It might be noted here that one of us, in Cincinnati, inoculated sterile milk with influenza sputum (1919) incubated it at 37°, 24°, and 15° for from 1 to 14 days, and fed it to white rats and white mice. Out of about 40 animals so fed, only 2 mice died—one with pneumococcus septicemia, and 1 with pneumonia and serous pleuritis, which was due to 4 different bacteria. None of these bacteria, singly or combined, produced infection when fed to other mice in milk or broth cultures.

TABLE II.—*Influenza—Sputum-spray experiments.*

Date.	Source of material used to infect.	Method of exposure.	Number and species of animals exposed.	Number of animals fatally infected.	Number infected animals with pneumonia.	Minimum and maximum duration of illness (days).	Percentage of fatalities.	Remarks.
1920.								
Feb. 3	Sputum S	Spray	5 mice	[1] 4	[2] 2	19–73	80	All like M–5.
Do	do	do	4 w. rats	2	[3] 2	13	50	Sterile.
Do	do	do	2 G. P	2	[4] 1	8–80	100	Like M–5.
Feb. 22	Lung emulsion M–1	Intraperitoneal	2 mice	2	0	6–8	100	General infection like M–5.
Feb. 23	Mixed culture M–1 plate	Spray	2 mice	2	2	8–12	100	M–5 like M–5.
Mar. 3	Lung emulsion M–5	do	2 mice	1	1	12	50	Like M–5.
Feb. 25	Organ emulsion M–2	do	2 mice	0	0		0	
Feb. 16	Organ emulsion W–1, W–2	Intraperitoneal	2 w. rats	0	0		0	
Apr. 23	Organ emulsion G. P. 3	Spray	2 mice	1	0	40	50	
Do	do	do	2 G. P	1	0	30	50	General infection like M–5.
Feb. 4	Sputum cultures case S	do	6 mice	2	2	31–45	33	No growth obtained from any tissue.
Do	do	do	2 G. P	0	0		0	Killed and found normal May 2, 1920.
Do	do	do	2 w. rats	0	0		0	
Do	do	do	1 rabbit	[6] 1		8	100	Gram positive coccus present in lung in large numbers.
Feb. 16	Culture R–1	Intravenously, intraperitoneal	2 rabbits	2	2	34–40	100	Infecting agent not R–1.
Mar. 2	Sputum R and S	Spray	2 mice	2	1	33–45	100	Like M–5.
Do	do	do	1 G. P	1	1	26	100	Do.
Mar. 3	Sputum B and B	do	8 mice	4	1	30–38	50	Like M–5. One sterile.
Apr. 4	Lung emulsion B and B, 30-day mouse	Intraperitoneal	2 mice	1	1	50	50	Like M–5.
Apr. 7	Lung emulsion B and B, 38-day mouse	do	1 mouse	1	0	3	100	Do.
Mar. 5	Sputum B and B	Spray	6 mice	1	0	52		Like M–5, from lung.
Do	Sputum T	do	6 mice	2	0	72–84	33	Like M–5.

[1] 1 killed. [2] M 1, M 2. [3] W 1, W 2. [4] G. P. 3. [5] M 5, M 6. [6] R 1.

RESULTS OF SPUTUM SPRAY EXPERIMENTS.

(*a*) *Family S., sputum spray.*—Four of the 5 mice exposed to the sputum spray of family S. died infected with a strain of *B. enteritidis* designated in this article as M–5. Two of these 4 mice died of a primary pneumonia which was due to M–5. By the term primary pneumonia we mean a pneumonia without a marked involvement of the liver and spleen, which invariably occurs in a general infection, e. g., after feeding.

Of 2 guinea pigs exposed to the sputum spray, one died with primary pneumonia caused by M–5. A normal saline extract was made of the spleen, liver, and lungs of this animal, and 2 mice and 2 guinea pigs were exposed to its spray. One of these mice and one guinea pig died of a general infection produced by M–5.

Four white rats were also exposed to the family S. sputum spray. Two of these rats died of a primary pneumonia. Lung cultures made from these animals yielded no growth. Organ extracts from these 2 rats were made and injected subcutaneously and intraperitoneally into other rats with negative results. Unfortunately a spray exposure was not done with the material from the rats.

As a control on (*a*), mixed cultures of the sputum used for spraying from family S. were made on rabbit's blood agar plates, incubating them for 24 hours at 37° C. under partial tension oxygen conditions. Six mice, 2 guinea pigs and 1 rabbit were exposed to a heavy spray of an emulsion made from this culture. Of these, 2 mice and 1 rabbit died of a primary pneumonia. Exhaustive cultural methods were used on these animals, but no bacteria like M–5 could be isolated. All cultures from the mice were sterile, and a gram positive coccus was the only organism isolated from the rabbit. The latter organism failed, however, to reproduce the disease in other rabbits. These animals were taken from the same lot as those used in the S. sputum spray experiments. These results were influential in leading to the conclusion, which is brought out later, that there is some infectious substance in the sputum or throat swabs of early cases of influenza which predisposes to later organized infection.

(*b*) *R and S sputum spray.*—Of the 2 mice and 1 guinea pig exposed to the spray, one mouse and the guinea pig died of a primary pneumonia produced by M–5. The remaining mouse died of a general infection caused by M–5.

(*c*) *B and B sputum spray of March 3, 1920.*—Of the 8 mice exposed to this sputum spray, one died of a pneumonia produced by M–5, and 2 died of an M–5 general infection.

(*d*) *B and B sputum spray of March 5, 1920.*—After the two-day interval, sputum was again collected from cases B and B, and 6 mice were exposed to its spray. Of these, 1 mouse died of a general infection due to M–5, but not until after a period of 2 months. The remaining mice were examined 3 months later and found to be normal.

(*e*) *Sputum spray, T.*—Six mice were exposed to this spray. Two died after 2 months; one had a general infection with M–5, the other mouse was normal except for congestion of the lungs. No bacteria could be detected. Upon examination after 3 months, the others of this lot were found to be normal and did not harbor M–5.

SUMMARY AND CONCLUSIONS OF SPUTUM SPRAY EXPERIMENTS.

The animals sprayed with influenza sputum comprised 33 white mice, of which 4 died of primary pneumonia and in addition to these 4 mice, there were 9 which died of a general infection with M–5. Out of this same lot of mice, 6 were sprayed with culture material, and of these, 2, which died, were sterile on bacteriological examination, 2 were killed and cultured two months later and found to be uninfected, and 2 were used for another experiment in which they survived for a month.

Of 5 guinea pigs exposed, 2 died of primary pneumonia. The lungs of these animals contained numerous *B. enteritidis*-like organisms which were culturally and serologically identical (agglutination and absorption) with M–5. Of 6 white rats similarly exposed, 2 died of a primary pneumonia due to an unrecognized cause and not transmissible to rats by subcutaneous or intraperitoneal inoculation.

The work of Krumwiede, Valentine, and Kohn (Jour. Med. Research, 1919, 39, p. 449) shows that these laboratory animals may develop spontaneous infection with members of the paratyphoid-enteritidis group. We did not encounter a single death among our unused stock due to such bacteria, nor were we able to isolate such bacteria from the intestinal tract, liver, spleen, or lungs of 6 normal mice. However, the experiments detailed below (Table VII) show that a certain number of mice which are intoxicated by killed cultures of M–5 or by the sterile Berkefeld filtrate of broth cultures develop a secondary infection with M–5. In such endogenous infections following intoxication, pneumonia occurred only twice out of 40 animals treated.

Furthermore, of 29 mice sprayed with a virulent culture of the pneumococcus, only 1 died of infection with M–5, and this mouse had received a previous dose of M–5 toxin.

On the other hand, as is shown later in Table V, mice exposed to spray cultures of M–5 almost invariably died of a primary pneumonia.

In the light of the above data one is tempted to believe that the animals developing infection with M–5 were injured in someway by something in the influenza sputum. Nevertheless the possibility of purely spontaneous infections exists, and the question can be settled only by further work with more adequate controls, i. e., an equal number of animals from each lot used for an experiment should have been kept under identical conditions as controls.

INOCULATION OF OTHER ANIMALS.

From one of the typical cases, R, 20 c. c. of blood was obtained. This was used to inoculate a series of animals not generally used in laboratory experiments, with the hope that a susceptible animal might be encountered. These were an 8-weeks-old pig, weighing about 100 pounds, a ferret, an opossum, a salamander, and a black-headed nun-bird. None showed any abnormal symptoms during 3 months' observation.

THE CULTURAL AND AGGLUTINATIVE RELATIONSHIP OF M–5.

A collection had been made of the *B. enteritidis*-like organisms isolated from animals dying from infection after they had been exposed to the sputum sprays. A preliminary comparative study was made of the members of this collection to see if they were the same organism as the original type isolated, designated M–5. It was found that there was exact correspondence between the organisms isolated, in regard to their agglutination, absorption of agglutinins, and cultural characteristics. The animals from which these strains were isolated came from several different lots; some were bought in the local markets, others were shipped from near-by towns, while about one-half the supply came from the stock of the Hygienic Laboratory, Washington, D. C.

It was thought opportune to make a study of this enteritidislike organism, comparing it in particular with the Danysz virus, the paratyphoids, and *B. enteritidis* itself. Since all the *B. enteritidis*-like organisms isolated from the mice and guinea pigs exposed to sputum sprays corresponded in their agglutination, absorption, and cultural characteristics, we used M–5 alone for the comparative study. Unfortunately only two antisera, M–5, with a titer of 1–800, and paratyphoid B., with a titer of 1–10,000, were available. The results of this test are given in Table III. It will be observed from this table that M–5 is entirely distinct from paratyphoid B., but that it is indistinguishable by this test alone from the Danysz virus and from *B. enteritidis*. However, the cultural results (Table IV) show that Danysz virus agrees with paratyphoid B. in its failure to ferment xylose, whereas M–5 agrees with *B. enteritidis* in the fermentation of this substance. This divergence was brought out by Krumwiede, et al., 1919. We have been helped also in this study by reference to the work of Jordan (Jour. Inf. Dis., 1920, 26, p. 427, and his preceding articles cited here) and that of Winslow, Kligler, and Rothberg (Jour. Bacteriology, 1919, 4, p. 429). The Danysz virus and paratyphoid B. were from the Hygienic Laboratory, and the *B. enteritidis* was of the Gaertner type and came from Prof. E. O. Jordan in 1901.

Five cultures of *B. enteritidis*-like organisms isolated from the stools of influenza patients by Sherwood, Downs, and McNaught (J. Inf. Dis., 1920, 26, p. 16) were kindly sent to us by Dr. Sherwood. None of these strains agglutinated with M–5 antiserum.

TABLE III.—*Simple agglutination of certain strains by B. paratyphoid B. and M–5 antisera, and the absorption of homologous agglutinins from the same.*

Strain.	Para B. antiserum.		M-5 antiserum.	
	Simple agglutination.	Homologous agglutinins absorbed.	Simple agglutination.	Homologous agglutinins absorbed.
M–5	−		+	+
Para B	+	+	+	−
Enteritidis	−		+	+
Danysz virus	−		+	+

EXPERIMENTS SHOWING THAT BROTH CULTURES OF M–5 CONTAIN A SOLUBLE TOXIN WITH WHICH AN ANTITOXIN MAY BE PRODUCED.

In working with broth cultures of M–5 it became evident that after the culture had been put through a Berkefeld N the filtrate contained a toxic substance. Plain, maltose, and dextrose beef infusion broths were tried out in an attempt to demonstrate this toxin. It was found that 0.1 per cent dextrose broth (+0.5) yielded the most potent toxin. After incubation at 37° C. for from 4 to 5 days the culture was filtered through a Berkefeld N. This filtrate would kill mice in from 12 to 18 hours when 0.05 to 0.1 c. c. was injected intraperitoneally. Seventy mice were used in establishing the nature and potency of this toxin. Mice dying of intoxication showed marked injection of the subcutis and congestion of the lungs. Often the pulmonary congestion bordered upon consolidation. The lungs often showed numerous capillary hemorrhages. Other organs and tissues appeared normal to the eye.

We found that about 2 c. c. of this toxin was the minimum lethal dose (M. L. D.), on intravenous inoculation, for a rabbit weighing 1,800 gms. By inoculation with sublethal doses and gradually increasing the amount at 2-day intervals, over a period of three weeks, a rabbit could tolerate 5 c. c. of a freshly prepared toxin. Eight days after the last dose of toxin the serum of this rabbit would protect mice when mixed with 2 M. L. D. of the toxin and at once inoculated intraperitoneally.

TABLE IV.—*The morphological and cultural characteristics of M–5 and of representative organisms selected from the same group.*

Strain.	Gram stain.	Motility.	Litmus milk. Initial acid.	Litmus milk. Final strongly alkaline.	Indol.	H_2S produced.	Gelatin liquified.	Voges proskauer.	Endo's acid.	Green colonies, partial lension blood agar plates.	Dextrose.	Maltose.	Galactose.	Saccharose.	Levulose.	Lactose.	Mannite.	Arabinose.	Dulcite.	Erythrit.	Isodulcite.	Mannose.	Raffinose.	Salacin.	Xylose.
M–5	-	+	+	+	-	+	-	-	-	+	ag	ag	ag	-	ag	-	ag	ag	ag	-	ag	ag	-	-	ag
Danysz virus	-	+	+	+	-	+	-	-	-	+	ag	ag	ag	-	ag	-	ag	ag	ag	-	ag	ag	-	-	ag
B. Para B	-	+	+	+	-	+	-	-	-	+	ag	ag	ag	-	ag	-	ag	ag	ag	-	ag	ag	-	-	-
Enteritidis	-	+	+	+	-	+	-	-	-	+	ag	ag	ag	-	ag	-	ag	ag	ag	-	ag	ag	-	-	ag

ag=acid and gas.

ANIMALS EXPOSED TO M–5 CULTURE SPRAY DEVELOP PNEUMONIA. SIMILAR RESULTS FOLLOW THE DANYSZ VIRUS SPRAY, WHEREAS NEGATIVE RESULTS ARE OBTAINED FROM A B. INFLUENZÆ SPRAY.

Inasmuch as the organism M–5 had been consistently found in the infected lungs of animals dying after exposure to the sputum sprays, an attempt was made to infect animals in a similar way by exposing them to a spray containing this strain in pure culture. Accordingly, 26 mice were exposed at various times to an M–5 culture spray. In connection with this experiment, 6 mice were exposed to a Danysz virus culture spray and 4 mice to *B. influenzæ* culture spray. This influenza culture used was a fresh one, being the first transfer from a culture isolated from a post-mortem lung 48 hours previously.

The results as recorded in Table V show that of 26 animals exposed to M–5 culture spray, 24 died of primary pneumonia in which the cause was the organism M–5. Five of 6 mice exposed to a culture spray of the Danysz virus died of primary pneumonia, whereas there were no fatalities and no illness among the group of mice exposed to a spray of a freshly isolated *B. influenzæ*.

TABLE V.—*Experiments showing that when animals are exposed to the spray of M–5 cultures, a large percentage develop pneumonia, and that similar results follow the spray of Danysz virus.*

Date.	Animals exposed.	Number of deaths.	Percentage dead with primary pneumonia.	Duration of illness in days.	Remarks.
1920					
Mar. 12	4 mice	4	100	4–20	
Mar. 17	10 mice	8	100	4–15	
Mar. 30	4 mice	4	75	1–10	Sublethal dose M–5 given few hours before spray. One toxin death.
Mar. 25	5 mice	4	100	4–12	
Apr. 8	1 mouse	1	0	16	
May 11	2 guinea pigs	2	100	11–17	
May 5	6 mice	5	100	12–20	Danysz virus spray.
Mar. 24	4 mice	0	0		*B. influenzæ*.

ATTEMPTS TO IMMUNIZE AGAINST M–5 CULTURE SPRAY BY INOCULATION WITH A KILLED CULTURE AND WITH SUBLETHAL DOSES OF M–5 SOLUBLE TOXIN.

As soon as it was known that the exposure of mice to M–5 culture spray produced a fatal primary pneumonia, attempts were made to immunize them against such infection. The data of the attempts to immunize with toxin are contained in Table VI. Four mice, controls, were exposed to the spray without any previous treatment. Of these, one, or 25 per cent, died. It was a typical M–5 pneumonia. The other 3 remained normal. Upon being chloroformed and examined 2 months later, no M–5 bacilli could be detected in their lungs.

TABLE VI.—*Experiments showing failure to immunize against M–5 culture spray by previous inoculations of sublethal doses of M–5 soluble toxin.*

Date.	Number of mice.	Number of deaths.	Percentage dead with primary pneumonia.	Duration of illness, in days.	Remarks.
1920.					
April 6	4	3	100	11-18	One dose soluble toxin 6 days before spray.
Do	5	4	100	12-44	Four doses soluble toxin at 3-day intervals. Last dose 5 days before exposure to spray.
Do	4	1	100	14	Controls without toxin.

Four mice were given 1 sublethal dose of M–5 toxin 6 days before exposure to the spray. Three of the 4 died in from 11 to 18 days with a typical M–5 pneumonia. Five mice were given 4 sublethal doses of M–5 soluble toxin at 3-day intervals, the last dose being given 5 days before the exposure to the culture spray. Four of the 5 mice died with a typical M–5 pneumonia. Apparently the normal fresh mice are not as susceptible to pneumonia following a short exposure to the M–5 culture spray as the mice that have been immunized with the toxin.

The data of the attempts to immunize with M–5 killed culture are included in Table VII. The vaccine was prepared by washing off an M–5 agar slant culture with normal saline and immediately killing by heating to 65° C. for one hour. The vaccine was then diluted to about the density of an ordinary 24-hour typhoid broth culture growth and preserved with 0.5 per cent phenol. Each mouse was given 0.1 c. c. subcutaneously at each dose. Three tests for sterility were made on the vaccine, one before it was used, one during use, and one after the vaccinations had been completed. It was found sterile at all times. There is no apparent difference between the vaccinated and nonvaccinated mice as far as infection with the spray is concerned. It is interesting to note that 2 of the mice in the series became infected (general infection) with M–5 after vaccination but previous to the exposure to the spray.

TABLE VII.—*Experiments showing failure to immunize against M–5 spray by previous subcutaneous inoculation with a killed culture.*

Date.	Number of mice.	Number of deaths.	Percentage dead with primary pneumonia.	Duration of illness in days.	Remarks.
1920.					
May 7	4	4	100	12-23	1 dose 10 days before exposure to spray.
Do	4	3	[1] 33	[1] 1- 8	2 doses at 4 days' interval. Last dose 10 days before spray.
Do	4	3	[1] 66	8-14	3 doses at 4 days' interval. Last dose 10 days before spray.
Do	5	5	80	6-14	Controls not vaccinated.

[1] Two mice died of general infection with M–5 before the time for exposure to the spray.

ATTEMPTS TO PRODUCE A PRIMARY PNEUMONIA IN MICE BY EXPOSING THEM TO M–5 CULTURE BY OTHER MEANS THAN THE SPRAY.

Having shown that mice are very susceptible to an M–5 primary pneumonia when they are exposed to its culture spray and that they are not protected from this pneumonia by the ordinary means of immunization, it seemed pertinent to determine whether or not this pneumonia could be produced by other means of exposure. The results obtained are shown in Table VIII.

TABLE VIII.—*Experiments showing that primary pneumonia is not produced in mice when inoculated with M–5 by other means than spray.*

Date.	Method of inoculation.	Number of mice.	Number of deaths.	Percentage dead with primary pneumonia.	Duration of illness in days.	Remarks.
1920.						
Mar. 18	Fed heavily	4	3	0	6–14	General infection.
Apr. 20	do	2	2	50	8–9	General infection; 1 with secondary pneumonia.
Do	do	2	2	0	12–17	General infection.
Do	Fed 5 drops	4	3	0	10–18	Do.
Do	Fed 1 drop	4	1	0	8	Do.
May 11	Subcutaneous	3	3	0	2–5	Do.
Mar. 31	Intraperitoneal	4	4	0	2–4	Do.
May 11	Ocular conjunctiva.	3	2	50	9–19	General infection; 1 with secondary pneumonia.

The mice fed M–5 were kept without food and water for from 1 to 2 days. Those fed drops of the culture were watched while they lapped up the drops of a 24-hour culture. Those fed heavily ate bread soaked with the broth culture. The mice injected subcutaneously were given minute portions of a young culture. Those inoculated in the ocular conjunctiva had loopfuls of the culture placed on the conjunctiva and in a similar way culture was placed in the nostrils of those infected by way of the anterior nares.

When the portal of entry was through the conjunctiva, the buccal and gastrointestinal mucosa, the subcutis or peritoneal cavity, general infection followed. In no case was there a primary pneumonia. In 2 cases there was a secondary pneumonia. In such cases the liver and spleen were greatly enlarged and full of whitish necrotic or proliferative areas. These organic lesions are not present in mice dying of inhalation pneumonia.

EXPERIMENTS SHOWING THAT SPRAYED BACTERIA ARE ACTUALLY INHALED INTO THE DEEPEST PARTS OF THE LUNGS.

When it was found that animals exposed to a spray of M–5 culture consistently developed a primary pneumonia, whereas those exposed by other means developed such an infection only very rarely, if at

all, it was considered advisable to attempt to demonstrate the presence of bacteria in the lungs after exposing the animals to such spray. Accordingly, a series of experiments was started. The results of these attempts follow.

(*a*) Four mice and 1 guinea pig were sprayed with a broth culture of M–5 and chloroformed within 30 minutes from the beginning of the experiment. They were wet with alcohol and immersed in 1: 1,000 bichloride of mercury solution for 5 minutes. Then they were dissected, with aseptic precautions, and from each animal 4 to 6 small pieces of the lungs (1 to 3 m.m. in diameter) were snipped off with sterile scissors and planted in broth. In every instance all the pieces yielded growth of M–5 within 24 hours. Many of the pieces of lung represented the extreme distal portions of the anterior and posterior lobes. Five normal mice controls were examined in the same way. All cultures from these remained sterile during 72 hours' observation.

(*b*) Experiment (*a*) was duplicated except that a very virulent pneumococcus (Type 1) was used and the tissue was planted in glucose rabbit blood broth. The 4 mice were killed and cultured as in experiment (*a*), with the exception that they were examined at 2, 4, 8, and 18-hour intervals after the spray. In every instance the cultures showed that pneumococci were present in the deepest parts of the lungs. Cultures from 1 control mouse yielded no growth in the same medium.

ATTEMPTS TO PRODUCE PNEUMONIA IN MICE BY SPRAYING PNEUMOCOCCI.

Having shown that sprayed bacteria reach the deepest alveoli, or capillary bronchi, of the lungs, and that pneumococci planted in this way survive in the lungs of mice for at least 18 hours, the maximum period tested, we made the following experiments with a type 1 pneumococcus. This culture had been kept highly virulent for mice at the Hygienic Laboratory, United States Public Health Service.

(*a*) Four mice were sprayed with the growth from 4 blood agar slants suspended in broth. They were exposed for 30 minutes. Two died of primary lobar pneumonia in 14 days. No bacteria could be found in the purulent exudate, and all cultures remained sterile. The remaining 2 were killed 6 weeks later. They appeared normal, and cultures from the lungs remained sterile.

(*b*) Ten mice were kept at 8° C. for 4 hours and then sprayed as under (*a*). They felt warm on removal from the ice box. Two were killed and cultured shortly after spraying. Pneumococci grew out of all pieces of their lungs including the most distal portions. At the end of 4 weeks the remaining 8 mice were chloroformed and cultured with negative results.

(*c*) Since M–5 soluble toxin injures the lungs of mice, 6 mice which had survived sublethal doses of this toxin given 10 days before were sprayed as under (*a*). They were killed 6 weeks later and found to be normal. Nor did they harbor pneumococci.

(*d*) Four mice were given sublethal doses of M–5 toxin and sprayed with pneumococci at once, as under (*a*). Six weeks later one of these mice died of pneumonia caused by M–5; no pneumococci could be found. The remaining 3 were killed and cultured 8 weeks later. They were normal and the cultures remained sterile.

(*e*) Three mice were sprayed, as under (*a*), with the pneumococcus and then, in an attempt to give them an acidosis, they were kept under ether for one hour. One of these was further chilled in ice water for 10 minutes. They survived and yielded no growths 4 weeks later.

(*f*) Two mice were sprayed with a broth suspension of bloody sputum from a case of pneumococcus lobar pneumonia. They remained well during 6 weeks' observation.

Since none of the 29 mice became infected after inhaling virulent pneumococci into their lungs, one may conclude that some predisposing factor must precede or accompany such an implantation of bacteria. While we owe the whole idea of droplet infection to Flügge and his pupils and confess that we have relied on the review of their work by Göetschlich (Handbuch d. path. mikroorg., Kolle and Wasserman, 2d. ed. Bd. 2) we are not aware whether these workers demonstrated the fact that bacteria are to be recovered from the deepest portions of the lungs of sprayed animals. Our attention was drawn to this by Rogers (Amer. Rev. Tuberc. 1919, 3, p. 238, and idem, 1920, 3, p. 750), of Cincinnati, who showed that tubercle bacilli could be recovered from the lungs of guinea pigs immediatly after spraying them with tuberculous sputum, and that such protected and sprayed animals develop true primary pulmonary tuberculosis.

We know the work of Dürck (Deutsh. Arch. f. Klin. Med. 1897, 58, p. 368) who, by means of intratracheal insufflation, was unable to infect the lungs of rabbits with freshly isolated cultures of pneumococcus, *streptococcus pyogenes*, and *staphylococcus aureus*, unless at the same time, or before or after, injurious dust particles, pumice, or "Thomasphosphatmehl" were also blown into the lungs. According to his work, sterile injurious dust produced pneumonia, whereas sterile street dust did not. He also describes the production of typical pneumonia in rabbits, with secondary invasion of the pneumonic areas by *B. coli*, Sarcinae, or Friedlander's bacillus, by keeping the rabbits at 37° to 41° C. for from 16 to 36 hours and then in ice water for from 2 to 7 minutes.

However, these experiments and those performed by the method of intrabronchial insufflation, which was introduced by Lamar and Meltzer (J. Exp. Med., 1912, Vol. 15) and used by many others in this country, do not appeal to us as representing what must take place under natural conditions. It is difficult to introduce infectious material by this method without so injuring the mucous membranes as to make the inoculation subcutaneous. Bacteria can be inhaled, however, into the deepest parts of the lungs; and if they are capable of multiplying there, they will produce pneumonia, as in the case of M–5. The fact that virulent pneumococci do not multiply when planted in the lungs of mice by air currents is an interesting fact and deserves further investigation.

SUMMARY AND CONCLUSIONS.

1. When white mice, white rats, and guinea pigs were exposed to finely divided influenza sputum sprays, some died of a primary pneumonia, others of a general infection that was due to a strain of *B. enteritidis* (Type M–5). Since the work of other investigators has shown that these animals may die of spontaneous infection with members of the paratyphoid-enteritidis group, we can not say that these infections were necessarily the sequel to the spray. However, as primary pneumonia could not be produced in mice when M–5 was inoculated through the buccal or gastrointestinal mucosa, the conjunctiva, subcutis, or peritoneal cavity, but only when sprayed, it seems to us likely that something in the sputum sprays produced a change in the pulmonary tissues favoring such secondary localization.

2. Broth cultures of M–5 contain a soluble toxin which produces marked congestion of the subcutaneous and pulmonary tissues of white mice. This toxin gives rise to an antitoxin when injected into rabbits. Previous inoculation with the toxin did not produce immunity to the development of primary pneumonia by sprayed cultures.

3. We were unable to immunize against the spray of M–5 cultures by previous subcutaneous inoculations with a dead culture.

4. The intoxication of mice with the soluble toxin or with killed cultures of M–5 apparently led to infection with M–5 in a small percentage of the used mice. We were not able to find this bacterium in normal mice, nor did spraying mice with virulent pneumococci make it show itself as a secondary invader.

5. Experiments show that M–5 and virulent pneumococci are inhaled by mice into the deepest alveoli or capillary bronchi of the lungs, and that primary pneumonia follows in the case of M–5 which is capable of growing and producing its toxin there, whereas the virulent pneumococci gradually disappear.